

## IN THE CLAIMS:

1. (Currently Amended) A method of preserving sperm using a cryoprotectant buffer comprising:
  - a) cooling a first sample solution which includes a sperm sample to a first temperature sufficient to protect sperm from glycerol toxicity, at a rate sufficiently slow that the metabolic rate of sperm is decreased, to provide a cooled first sample solution, maintained for a first period of time at said first temperature;
  - b) adding a solution comprising glycerol to said cooled first sample solution after said first period of time;
  - c) mixing said glycerol and said cooled first sample solution together to comprise a second sample solution;
  - d) lowering the temperature of said second sample solution to a second temperature for a sufficient period of time to equilibrate glycerol and sperm;  
and
  - e) freezing said second sample solution to thereby provide a frozen sperm sample, such that the sperm is preserved;  
  
wherein said first sample solution does not contain glycerol;  
wherein said second sample solution further comprises an antibiotic compound;  
wherein said sperm sample is cooled to said ~~first~~ second temperature and maintained at said ~~first~~ second temperature for 4 hours to 21 hours so as to allow the equilibration of glycerol with said sperm sample;  
wherein said second sample solution is allowed to freeze at said second temperature.
3. (Previously Presented) The method of claim 1, wherein said second sample solution comprises about 10% to about 30% egg yolk.

4. (Previously Presented) The method of claim 1, wherein said second sample solution comprises 5% to about 10% glycerol.
5. (Previously Presented) The method of claim 1, wherein said sperm sample is obtained from a mammal.
6. (Previously Presented) The method of claim 1, wherein said first temperature is between 0°C and 10°C.
8. (Previously Presented) The method of claim 1, wherein said first sample solution is cooled at a rate of between 0.2°C and 0.5°C per minute to reach said first temperature.
9. (Previously Presented) The method of claim 1, wherein said first sample solution is cooled to the first temperature over the course of 1.5 to 4 hours.
10. (Previously Presented) The method of claim 1, wherein said second temperature is between -40°C and -100°C.
11. (Previously Presented) The method of claim 1, wherein the sample is maintained at said second temperature for between 7 minutes and 20 minutes.
12. (Previously Presented) The method of claim 1, wherein the method further comprises storing the frozen sperm sample at a third temperature of between -190°C and -200°C.
13. (Currently Amended) A method of preserving sperm comprising:
  - a) combining a sperm sample with a first cryoprotectant buffer to form a first cryoprotectant solution;

- b) cooling said first cryoprotectant solution to a first temperature such first temperature being between 2°C and 10°C at a rate sufficiently slow that the metabolic rate of sperm is decreased to produce cooled sperm;
- c) freezing said first cryoprotectant solution by lowering the temperature of said first cryoprotectant solution to a second temperature between -60°C and -90°C ~~wherein said first cryoprotectant solution can freeze~~; and
- d) storing said frozen first cryoprotectant solution in liquid nitrogen at a third temperature;

wherein said sperm sample is cooled to said first temperature and maintained at said first temperature for 4 hours to 21 hours so as to allow the equilibration of glycerol with said sperm sample;

wherein said first cryoprotectant solution further comprises an antibiotic compound[.];

wherein said first cryoprotectant buffer comprises 5% to 10% glycerol weight to volume of said cryoprotectant buffer; and,

wherein said first cryoprotectant solution is further comprised of egg yolk, fructose, citric acid, Tris buffer; and an antibiotic compound.

**Claims 14 - 15. (cancelled)**

- 16. (Previously Presented) The method of claim 13, wherein said sperm sample is obtained from a mammal.
- 17. (Previously Presented) The method of claim 13, wherein said sperm sample is maintained at said first temperature for between 4 hours and 21 hours.

18. (Previously Presented) The method of claim 13, wherein said first cryoprotectant solution is cooled at a rate of between 0.2°C and 0.5°C per minute to reach said first temperature.
19. (Previously Presented) The method of claim 13, wherein said first cryoprotectant solution is cooled to said first temperature over the course of 1.5 hours to 4 hours.
20. (Previously Presented) The method of claim 13, wherein a second cryoprotectant buffer is added to said first cryoprotectant solution after said first cryoprotectant solution has been cooled to said first temperature, but before said first cryoprotectant solution has been further cooled to said second temperature or frozen.
21. (Previously Presented) The method of claim 20 wherein said second cryoprotectant buffer comprises 5% to 10% glycerol.
22. (Previously Presented) The method of claim 13, wherein said second temperature is at least -80°.
23. (Previously Presented) The method of claim 13, wherein said first cryoprotectant solution is maintained at said second temperature for between 7 minutes and 20 minutes.
24. (Previously Presented) A method of preserving sperm comprising:
  - a) providing a sample comprising sperm;
  - b) isolating sperm from said sample;
  - c) combining said isolated sperm with a first cryoprotectant buffer to form a first sample solution;

- d) cooling said first sample solution to a first temperature between 2°C and 8°C at a rate of between 0.2°C and 0.5°C per minute to produce said first sample solution at said first temperature;
- e) adding a second cryoprotectant buffer comprising glycerol to form a second sample solution;
- f) maintaining said second sample solution at said first temperature for a period of between 4 hours and 21 hours;
- g) lowering the temperature of said second sample solution by cooling said second sample solution to a second temperature of between -60°C and -90°C for a time of between about 10 minutes to about 15 minutes;
- h) allowing said second sample solution to freeze at said second temperature;
- i) storing said second sample solution at a third temperature of between -180°C and -220°C for a desired time period;
- i) thawing said sperm, to thereby provide sperm for use; and

wherein said first sample solution does not contain glycerol;

wherein said second sample solution further comprises an antibiotic compound.

- 25. (Previously Presented) The method of claim 24, wherein the sperm are thawed for about 90 seconds in a water bath at about 37°C prior to use.
- 26. (Previously Presented) A method of making an animal, comprising fertilizing an oocyte with sperm preserved by the method of claims 1, 13, or 24.
- 27. (Currently Amended) A method of preserving sperm comprising:

- a) cooling a first sample solution which includes sperm to a first temperature ~~sufficient to protect sperm from glycerol toxicity~~, at a rate sufficiently slow that the metabolic rate of sperm is decreased, to provide a cooled first sample solution, maintained for a period of time at said first temperature;
- b) adding a cryoprotectant solution to said cooled first sample solution;
- c) mixing said cryoprotectant solution and said cooled first sample solution together to comprise a second sample solution;
- d) lowering the temperature of said second sample solution to a second temperature for a sufficient period of time to equilibrate said cryoprotectant solution and sperm comprising said second sample solution; and
- e) freezing said second sample solution to thereby provide a frozen sperm sample, such that the sperm is preserved;

wherein said cryoprotectant solution is further comprised of egg yolk, fructose, citric acid, Tris buffer; and an antibiotic compound;

wherein said sperm sample is cooled to said first temperature and maintained at said first temperature for 4 hours to 21 hours[.];

wherein neither said first sample solution or said second sample solution contains glycerol;

wherein said egg yolk comprises 10% to 30% weight to volume of said cryoprotectant solution; and,

wherein said citric acid comprises at least 1.5% weight to volume of said cryoprotectant solution;

wherein said fructose comprises at least 1% weight to volume of said cryoprotectant solution.

**Claims 28 – 29 (Cancelled)**

30. (Previously Presented) The method of claim 27 wherein said egg yolk comprises 20% weight to volume of said cryoprotectant solution.

**Claims 31 – 32 (Cancelled)**

33. (Currently Amended) The method of claims 1, 13, 24 or 27 wherein said antibiotic compound is selected from a group consisting of:
- a) tylosin;
  - b) gentamicin;
  - c) lincospectin; and
  - d) spectinomycin.

**Please Cancel Claims 34-44**

**Claims 34 – 44 (cancelled).**